

Corresponding author(s):	Zhiqian Tong
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Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see <u>Authors & Referees</u> and the <u>Editorial Policy Checklist</u>.

Statistics		
	es, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.	
n/a Confirmed	,,,,	
	uple size (n) for each experimental group/condition, given as a discrete number and unit of measurement	
	on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly	
The statistical	test(s) used AND whether they are one- or two-sided ests should be described solely by name; describe more complex techniques in the Methods section.	
A description	of all covariates tested	
A description	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons	
A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)		
	hesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted exact values whenever suitable.	
For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings		
For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes		
Estimates of e	ffect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated	
'	Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.	
Software and c	ode	
Policy information abou	ut <u>availability of computer code</u>	
Data collection	Discovery Studio 3.0	
Data analysis	PyMOL 1.7 software	
	om algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.	
Data		
- Accession codes, un - A list of figures that	ut <u>availability of data</u> include a <u>data availability statement</u> . This statement should provide the following information, where applicable: ique identifiers, or web links for publicly available datasets have associated raw data restrictions on data availability	
A list of figures that have	associated raw data and we will providence the raw data as soon as the requirement.	
Field-speci	fic reporting	
Please select the one b	elow that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.	
Life sciences	Behavioural & social sciences 💢 Ecological, evolutionary & environmental sciences	

For a reference copy of the document with all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>

Ecological, evolutionary & environmental sciences study design All studies must disclose on these points even when the disclosure is negative.

Ct		
Study description	Briefly describe the study. For quantitative data include treatment factors and interactions, design structure (e.g. factorial, nested, hierarchical), nature and number of experimental units and replicates.	
Research sample	Describe the research sample (e.g. a group of tagged Passer domesticus, all Stenocereus thurberi within Organ Pipe Cactus National Monument), and provide a rationale for the sample choice. When relevant, describe the organism taxa, source, sex, age range and any manipulations. State what population the sample is meant to represent when applicable. For studies involving existing datasets, describe the data and its source.	
Sampling strategy	Note the sampling procedure. Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient.	
Data collection	Describe the data collection procedure, including who recorded the data and how.	
Timing and spatial scale	Indicate the start and stop dates of data collection, noting the frequency and periodicity of sampling and providing a rationale for these choices. If there is a gap between collection periods, state the dates for each sample cohort. Specify the spatial scale from which the data are taken	
Data exclusions	If no data were excluded from the analyses, state so OR if data were excluded, describe the exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.	
Reproducibility	Describe the measures taken to verify the reproducibility of experimental findings. For each experiment, note whether any attempts to repeat the experiment failed OR state that all attempts to repeat the experiment were successful.	
Randomization	Describe how samples/organisms/participants were allocated into groups. If allocation was not random, describe how covariates were controlled. If this is not relevant to your study, explain why.	
Blinding	Describe the extent of blinding used during data acquisition and analysis. If blinding was not possible, describe why OR explain why blinding was not relevant to your study.	
Did the study involve field	d work? Yes No	
Field conditions	Describe the study conditions for field work, providing relevant parameters (e.g. temperature, rainfall).	
Location	State the location of the sampling or experiment, providing relevant parameters (e.g. latitude and longitude, elevation, water depth).	
Access and import/expo	Describe the efforts you have made to access habitats and to collect and import/export your samples in a responsible manner and in compliance with local, national and international laws, noting any permits that were obtained (give the name of the issuing authority, the date of issue, and any identifying information).	
Disturbance	Describe any disturbance caused by the study and how it was minimized.	
Ve require information from	or specific materials, systems and methods authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, evant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.	
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/e require information from /stem or method listed is related in the study /a Involved in the study Antibodies Eukaryotic cell lines	authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, evant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response. Methods	

Palaeontology		
	Provide provenance information for specimens and describe permits that were obtained for the work (including the name of the issuing authority, the date of issue, and any identifying information).	
Specimen deposition	Indicate where the specimens have been deposited to permit free access by other researchers.	
	If new dates are provided, describe how they were obtained (e.g. collection, storage, sample pretreatment and measurement), where they were obtained (i.e. lab name), the calibration program and the protocol for quality assurance OR state that no new dates are provided.	
Tick this box to confirm that	at the raw and calibrated dates are available in the paper or in Supplementary Information.	
ChIP-seq		
Data deposition		
Confirm that both raw and	final processed data have been deposited in a public database such as <u>GEO</u> .	
Confirm that you have dep	osited or provided access to graph files (e.g. BED files) for the called peaks.	
Data access links May remain private before publication.	For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.	
Files in database submission	Provide a list of all files available in the database submission.	
Genome browser session (e.g. <u>UCSC</u>)	Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.	
Methodology		
Replicates	Describe the experimental replicates, specifying number, type and replicate agreement.	
Sequencing depth	Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.	
Antibodies	Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.	
Peak calling parameters	Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.	
Data quality	Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.	
Software	Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.	
Flow Cytometry		
Plots		
Confirm that: The axis labels state the many states are the many states.	arker and fluorochrome used (e.g. CD4-FITC).	
The axis scales are clearly v	visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).	
All plots are contour plots	with outliers or pseudocolor plots.	
A numerical value for number of cells or percentage (with statistics) is provided.		
Methodology		

Sample preparation Describe the sample preparation, detailing the biological source of the cells and any tissue processing steps used.

Instrument *Identify the instrument used for data collection, specifying make and model number.*

Software Describe the software used to collect and analyze the flow cytometry data. For custom code that has been deposited into a

community repository, provide accession details.

Cell population abundance

Describe the abundance of the relevant cell populations within post-sort fractions, providing details on the purity of the samples and how it was determined.

Gating strategy

Describe the gating strategy used for all relevant experiments, specifying the preliminary FSC/SSC gates of the starting cell population, indicating where boundaries between "positive" and "negative" staining cell populations are defined.